

# Methods to Identify and Characterize Developmental Neurotoxicity for Human Health Risk Assessment: Overview

Beth E. Miles<sup>1</sup> and Susan A. Ferenc<sup>2</sup>

<sup>1</sup>ARCADIS Geraghty and Miller, Millersville, Maryland, USA; <sup>2</sup>Grocery Manufacturers of America, Washington, DC, USA

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In 1991, the U.S. Environmental Protection Agency (U.S. EPA) issued a standardized protocol for evaluation of developmental neurotoxicity (DNT) for use in human health risk assessment (1). Briefly, the protocol comprises *a*) tests for evidence of deficits in neurobehavioral functions including auditory startle, habituation, associative learning and memory; *b*) monitoring of motor activity; and *c*) neuropathologic examination including simple morphometric analysis. An analysis of 12 developmental neurotoxicity studies submitted to the U.S. EPA led to identification of several issues to be considered in an effort to improve the protocol (2,3). These issues included the appropriate route of neurotoxicant administration; the duration of treatment and dosing levels of pups; potential use of pharmacokinetic data to support developmental neurotoxicity studies; the role and efficacy of morphometric data in evaluation of developmental neurotoxicity; the efficacy of current studies in evaluating age-related susceptibility; interpretation of behavioral data; and identification of biologic marker(s) of pup exposure and/or effect.

The U.S. EPA intends to revise the Office of Prevention, Pesticides and Toxic Substances developmental neurotoxicity guidelines in the near future and expects to address the above issues in the process. In addition, the Organisation for Economic Co-operation and Development has been refining new guidelines for DNT testing (4). In this context, these and other scientific issues important to characterizing developmental neurotoxicity for human health risk assessment must be considered. The International Life Sciences Institute (ILSI) Risk Science Institute (RSI) (5) entered into a cooperative agreement with the Office of Pesticide Programs of the U.S. EPA to address some of the difficult issues listed above. RSI convened a working group of experts (6) in developmental neurobiology, neuropathology, neurotoxicology, and neuropharmacology from government, industry, academia, and the public interest sector to consider the scientific basis of the aforementioned issues.

## Summary

Developmental neurotoxicity is any effect of a toxicant on the developing nervous system

before or after birth that interferes with normal nervous system structure or function. Developmental neurotoxicology is both a basic science, wherein toxicants are used as tools to increase our understanding of developmental neurobiology, and an applied science, wherein data are generated to address questions about risk and sensitivity. The latter neurotoxicology studies are conducted to identify or characterize potential developmental neurotoxicity of specific chemicals on laboratory animals, and the data are then used in human health risk assessment. Risk assessment of a developmental neurotoxicant requires careful consideration of the end point of toxicity, the dose–response relationship, and the relevance of the animal model to humans. This might require data from behavioral, neuropathologic and pharmacologic components of developmental neurotoxicity studies. The methods and techniques used in developmental neurotoxicology studies are being continually refined and will depend on the questions to be addressed and the goals of the study. This introductory article sets the stage for the three following articles that describe methods used to characterize developmental neurotoxicity, the state of the science of applied developmental neurotoxicology, and the strengths and weaknesses of various methods and techniques.

State-of-the-science developmental neurotoxicology studies focus on the effects of toxicants on particular structures and functions of the developing nervous system, using laboratory animals as a surrogate for the human. The mammalian central and peripheral nervous systems are complex structures resulting from essential developmental processes including cell proliferation, differentiation, migration, synaptogenesis, and myelination [for example, see Bayer et al. (7) and Rice and Barone (8)]. These stages of development normally occur sequentially according to different temporal profiles within specific regions of the brain and result in a heterogeneous pattern of synaptic connectivity. Integration of the connections formed and stabilized during brain development depends on a process of refinement and selective consolidation of the

neural circuitry. This process begins during gestation and continues postnatally through adolescence. Critical windows of vulnerability in the developing nervous system must be considered in neurotoxicity testing (9). These critical windows are dependent, for example, on the developmental stage of the target tissue or nervous system component, the mechanism of action of the toxic agent, the dose of the agent delivered to the target tissue, the toxic end point of interest, the age of the offspring during testing, and the method used to evaluate the outcome. The desire of investigators to maximize the likelihood of detecting an adverse effect on the nervous system in toxicity studies has led an impetus to evaluate multiple end points of toxicity at multiple times and dosing levels during pre- and postnatal development. However, an attempt to account for all possible end points and windows of vulnerability in a single experiment can lead to an unmanageable experimental design. The potential problems associated with an overly complex experimental design must be weighed against the possibility that inadequate evaluation might fail to identify a significant developmental neurotoxicant effect.

The ILSI RSI working group was asked to identify and describe sensitive yet practical methods to detect and/or characterize developmental neurotoxicity in laboratory animals. The working group separated into

Address correspondence to B.E. Miles, ARCADIS Geraghty & Miller, 1131 Benfield Blvd., Suite A, Millersville, MD 21108 USA.

Address reprint requests to S. Carter, ILSI Risk Science Institute, 1126 16th St. NW, Washington, DC 20036 USA. Telephone: (202) 659-3306. Fax: (202) 659-3617. E-mail: [scarter@ilsi.org](mailto:scarter@ilsi.org).

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three breakout groups, each to focus on a specific area in their discussions of developmental neurotoxicity: behavioral testing, neuropathology, and pharmacokinetics. Several of the key scientific and technical challenges to evaluation of developmental neurotoxicity addressed by the breakout groups are summarized below.

The breakout group on behavioral testing considered the alterations in nervous system function that may be identified in laboratory animals, using neurobehavioral methods (10). Careful consideration in experimental design is key to the success of studies that rely on behavioral methods to evaluate developmental neurotoxicity. Neurobehavioral assessment methods are used routinely to evaluate the effects of developmental neurotoxicants on sensory function, motor function, and cognitive function. Guiding principles for proper use and interpretation of neurobehavioral methods are essential for scientists who conduct or evaluate developmental neurotoxicity studies. Fundamental issues that underlie proper use and interpretation of these methods include *a*) consideration of the scientific goal in experimental design, *b*) selection of an appropriate animal model, *c*) the expertise of the investigator, *d*) adequate statistical analysis, and *e*) proper data interpretation. The appropriate behavioral test methods are selected on the basis of study goals and objectives. Considerations in method selection include the sensitivity required to detect a given effect, the selectivity required to identify or characterize an effect, available resources, and the inherent variability in the methods.

The neuropathology breakout group addressed the approaches used in morphologic and histopathologic assessment of toxicant-induced alterations in the developing nervous system for human health risk assessment (11). Morphologic assessment of the developing brain requires an understanding of corresponding timeframes for the critical events in nervous system development of the rat and human. This global spatial-temporal map, together with an understanding of the pathogenesis of nervous system malformations, can guide the pathologist in the identification and interpretation of toxicant-related effects (6). Two considerations are central to the morphologic identification of adverse effects. One is that normal developmental events such as programmed cell death must be distinguished from treatment-related effects that may exhibit similar morphologic

characteristics. A second consideration is that disruption of relatively early developmental events can alter growth and differentiation, thus resulting in alterations in the size of neuroanatomic structures. Quantitative morphologic evaluation of developmental neurotoxicant effects can include a variety of techniques, from simple determination of brain weight and dimensions, to the progressively more complex approaches of linear, areal, or stereologic measurement of brain and peripheral nervous system sections. Histologic evaluation employs routine stains (such as hematoxylin and eosin) that can be complemented by a variety of special staining and immunohistochemical procedures. These brain studies are augmented by morphologic assessment of selected peripheral nervous system structures. Studies of this nature require a high level of technical skill, as well as special training on the part of the pathologist.

The breakout group on pharmacologic testing agreed that pharmacokinetic and pharmacodynamic factors should be considered in the design and interpretation of developmental neurotoxicity studies (12). Many of the pharmacokinetic methods, analytical chemical techniques, and modeling approaches needed to evaluate the pharmacokinetics of a developmental neurotoxicant in the developing organism are available to the research investigator, though they are not routinely used in applied studies. Pharmacologic data may allow an experimenter to determine whether exposure to the test chemical is adequate, whether exposure occurs during critical periods of nervous system development, whether route and duration of exposure are appropriate, and whether developmental neurotoxicity can be differentiated from pharmacologic effects. Physiologically based pharmacokinetic and pharmacodynamic models can be used to estimate tissue dose and biologic response following *in utero* or neonatal exposure. Application of these models can reduce uncertainty in the extrapolation of results from animal studies to humans.

The following three consensus articles summarize the discussions and conclusions of the breakout groups. Methods and techniques to evaluate the behavioral and pathologic effects of exposure to toxicants have been used for many years to characterize developmental neurotoxicity [see U.S. EPA guidelines for example (1)]. The pharmacologic methods now being considered have shorter history of use in the design and interpretation of developmental neurotoxicity studies and

are less well established. Consequently, the articles on behavioral testing and neuropathology focus on proper use of current methods and techniques, whereas the article on pharmacokinetics focuses on incorporation of new methods in developmental neurotoxicity testing. These articles should inform scientists in general and guide professionals who are not necessarily specialists in all aspects of developmental neurotoxicology.

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5. ILSI is a nonprofit, worldwide foundation established in 1978 to advance the understanding of scientific issues relating to nutrition, food safety, toxicology, risk assessment, and the environment by bringing together scientists from academia, government, industry, and the public sector to solve problems with broad implications for the well-being of the general public.
6. Working Group members are Sandra Allen, Januz C. Byczkowski, Luz Claudio, Deborah A. Cory-Slechta, Kevin M. Crofton, David C. Dorman, J. Edward Fisher, Jeffrey W. Fisher, Andrew S. Fix, Jeffrey A. Foran, Robert H. Garman, Jerry F. Hardisty, G. Jean Harry, Karl Jensen, Bernard S. Jortner, Abby A. Li, Susan L. Makris, Stephanie Padilla, Joseph F. Ross, Larry P. Sheets, Lester G. Sultatos, Bernard Weiss.
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